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Minutes

Agricultural Biotechnology Research Advisory Committee

June 29–30, 1993

Document No. 93-01

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL BIOTECHNOLOGY RESEARCH ADVISORY COMMITTEE
Minutes of Meeting
June 29-30, 1993

Time, Place, and Participants

The United States Department of Agriculture (USDA) Agricultural Biotechnology Research Advisory Committee (ABRAC) met June 29-30, 1993, in the Board of Directors Room, North Carolina Biotechnology Center, 15 T.W. Alexander Drive, Research Triangle Park, North Carolina. The meeting had been announced in the *Federal Register* of May 20, 1993 (58 FR 29384), and it was open to the public.

Members present included:

David Kline, Chair, State University of New York, New Paltz, NY;
Anne Vidaver, Vice Chair, University of Nebraska, Lincoln, NE;
William Witt, Food and Drug Administration, National Center for Toxicological Research, Jefferson, AR;

David Andow, University of Minnesota, St. Paul, MN;
Stanley Pierce, Rivkin, Radler & Kremer, Uniondale, NY;
Walter Hill, Tuskegee University, Tuskegee, AL;

Anne Kapuscinski, University of Minnesota, St. Paul, MN;
Susan Harlander, Land O'Lakes, Inc., Minneapolis, MN;
James Lauderdale, Upjohn Company, Kalamazoo, MI;

Pamela Marrone, Novo Nordisk Entotech, Inc., Davis, CA;
Deborah Letourneau, University of California, Santa Cruz, CA;
Rudy Wodzinski, University of Central Florida, Orlando, FL;

Alvin Young, Executive Secretary, ABRAC, and Director, USDA Office of Agricultural Biotechnology, Washington, DC.

USDA Office of Agricultural Biotechnology (OAB) staff present included: Daniel Jones, Maryln Cordle, Martha Steinbock, and Marti Asner. Others present are listed in Appendix A.

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Call to Order and Approval of Agenda

Dr. Kline called the meeting to order at 3:05 p.m. He introduced Dr. Rudy Wodzinski who was attending as an ABRAC member for the first time. At the request of Dr. Thomas Hoban and Dr. Sally Van Wert, Dr. Kline changed the time of their presentations from

Wednesday afternoon to Wednesday morning and the ABRAC approved the agenda as modified.

Presentation of Transgenic Animal Working Group Report

Dr. Kline invited Dr. Lauderdale to present the report of the ABRAC Transgenic Animal Working Group to the full ABRAC. Dr. Lauderdale summarized the charge to the Working Group as a request to the ABRAC from the USDA Food Safety and Inspection Service (FSIS) to address the scientific questions associated with the human food safety of food products prepared from transgenic animals.

Dr. Lauderdale acknowledged the efforts of his fellow Working Group members: Dr. William Witt, FDA National Center for Toxicological Research; Dr. Bennie Osburn, University of California, Davis; Dr. Susan Harlander, Land O'Lakes, Inc.; Dr. Ann Boyd, Hood College; Dr. Richard Witter, USDA/ARS Poultry Research Laboratory; Dr. Harold Hafs, Merck, Sharp, & Dohme Research Laboratories; Dr. Gary Weber, USDA Extension Service; and Dr. Duane Kraemer, Texas A&M University.

Dr. Lauderdale directed the Committee's attention to draft document 195a entitled "Recommendations of the Transgenic Animal Working Group to the ABRAC on the Process to Assess Human Food Safety of Transgenic Animals, 29 June 1993" (Appendix B). He reported that the Working Group members had concurred in this draft. He proposed to talk through the draft point-by-point and to entertain suggestions for revision from the full ABRAC.

Dr. Lauderdale referred to the criteria for nontransgenic animals outlined in the FSIS "Decision Criteria for the Evaluation of Nontransgenic Animals from Transgenic Animal Research" (Appendix C). Those criteria were: 1) failure to detect the presence of the transgene by Southern hybridization, the polymerase chain reaction, or other appropriate scientific methods, 2) absence of measurable gene product, 3) absence of transgene-associated traits, and 4) a healthy appearance.

Dr. Lauderdale proposed that these criteria for non-transgenic animals be adapted to criteria for transgenic animals as follows: 1) detection of the transgene by Southern hybridization, polymerase chain reaction, or other appropriate methods, or 2) presence of measurable gene product, or 3) presence of transgene-associated traits. The level of detection for the transgene proposed by the Working Group was one copy of the foreign DNA per haploid genomic equivalent by DNA hybridization techniques. Dr. Lauderdale suggested that the appearance of the animal, other than the presence of transgene-associated traits, would not be so important as a criterion for transgenic animals.

Dr. Lauderdale summarized the Working Group's current thinking for

evaluating the human food safety implications of gene products (endogenous and non-endogenous), amplifications and deletions, mosaic animals, "naked" DNA, marker genes, DNA chemical modifications, vaccines, viral vectors, antisense nucleic acids, and somatic cell therapy (Document #195a, Appendix B).

Dr. Lauderdale referred to the definition of transgenic animal in Document #195a, p. 3. He proposed adding the following sentence to the definition: "The genetic composition of transgenic animals has been changed by introducing specific genes (e.g., recombinant DNA) from exogenous sources." He expressed an intent to exclude animals produced by traditional animal breeding from this definition.

Committee Discussion of Transgenic Animal Working Group Report

Dr. Andow asked what the Working Group considered to be traditional animal breeding. Dr. Lauderdale replied that the Working Group discussed this question and a multiplicity of perspectives were expressed. Dr. Andow questioned why an exclusion for traditional animal breeding was necessary. Dr. Lauderdale replied that if traditional animal breeding were not excluded, it might come under regulation without a thorough public discussion of the reason or necessity for doing so. Dr. Kline suggested that traditional animal breeding could be excluded in the preamble rather than in the definition.

Dr. Vidaver asked about the impact of transgenic animal regulation on animal science research. Dr. Lauderdale replied that it should be no greater than that of current human food safety regulations and guidelines. He added that FSIS has the option of addressing traditional animal breeding, animal science research, and related topics in the preamble to its transgenic animal policy document when it is prepared for publication in the *Federal Register*.

Dr. Andow expressed concern about the coverage of endogenous genes and he requested clearer definitions of "endogenous" and "exogenous." Dr. Lauderdale proposed defining "exogenous" as either "from outside the individual animal" or "from outside the animal line." He then argued that transfer of a pig gene into a pig was not really transfer of foreign genetic material and this would support the animal line as the logical boundary between endogenous and foreign.

Dr. Flamm, Food and Drug Administration, argued that the question of whether the DNA is endogenous or foreign is irrelevant. He suggested that transgenic animals, for the purpose of this document, are animals and their progeny in which the genetic composition of the animals has been changed by introducing either specific genes or gene segments by nontraditional methods.

In place of "exogenous DNA," Dr. Lauderdale suggested the phrase "DNA segments introduced by non-traditional methods." Dr. Basu,

FSIS, expressed concern that what may be considered non-traditional today may not be considered non-traditional in the future.

Dr. Wodzinski suggested that the definition of transgenic animal exclude amplification and deletion so that it would more closely resemble the concept of biological species which could then be used to delineate endogenous from foreign. Dr. Letourneau expressed doubt that she could agree with that proposal. Dr. Pierce suggested that a simple definition of foreign DNA would be nonparental. He expressed concern that further elaborations of the definition would make it too long. Dr. Andow argued that "transgenic" implied the movement of genes from one animal to another and that the definition should capture both interspecific and intraspecific gene transfers.

Dr. Wodzinski asked if every animal would be tested to determine if it were transgenic. Dr. Lauderdale replied that initially, every animal would be tested. He added that after sufficient experience is gained with transgenic animals, it may be possible to use a transgene-linked marker gene that would be easier to detect.

Dr. Flamm advanced the view that there is no reason to distinguish between endogenous and exogenous genes. He argued that the definition should not imply that the source of a gene determines its safety because, in his view, that idea is specious. He recommended that the definition of transgenic animal should not include intraspecific gene transfers, and that the distinction between endogenous and exogenous genes should be deleted.

Dr. Kapuscinski and Dr. Vidaver countered that intraspecific gene transfers with novel combinations of structural genes and regulatory elements can lead to significant consequences such as gene amplification, increases in the level of gene expression, and expression in new tissues. Dr. Marrone added that evaluating the safety of a product implies considering the source of the new genetic material. Dr. Lauderdale added that endogenous genes generally have a history of safe expression in the host while exogenous genes may result in the expression of a new gene product of unknown consequence for safety. Dr. Andow expressed support for Dr. Lauderdales's terminology in preference to the endogenous/exogenous distinction.

Dr. Flamm conceded that newness or novelty of gene expression may be important for safety, but he reiterated his point that the endogenous/exogenous distinction is not important for risk assessment. He added that an animal that is alive and well is its own test for safety.

Dr. Lauderdale recommended collapsing the endogenous/exogenous sections of the Working Group report into a single category for gene product and the Committee concurred.

Committee members discussed additional revisions of Document 195a on secondary influences, "naked" DNA, marker genes, chemically modified or synthetic nucleic acids, and gene amplification and deletions. Dr. Kline asked Dr. Lauderdale, Dr. Kapuscinski, Dr. Harlander, and Dr. Vidaver to work on revisions to the sections on viral vectors, mosaic animals, antisense nucleic acids, and somatic cell therapy, and to report back to the full Committee on the following day. Dr. Kline recessed the meeting until the following day.

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Dr. Kline reconvened the meeting at 9:11 a.m. the following day. He invited Dr. Sally Van Wert, Animal and Plant Health Inspection Service (APHIS), to update the Committee on the APHIS notification rule.

APHIS Notification Rule

Dr. Van Wert reported on the final rule covering introductions of genetically engineered plants and microorganisms which may present a plant pest risk. The final rule was published in the *Federal Register* of March 31, 1993 (58 FR 17044) and incorporated public comments received on an earlier proposed rule. The new rule amended the plant pest regulations (7 CFR 340) in two areas: provision of a notification process in place of a permit application process for certain plants with a substantial history of field testing (tobacco, tomato, potato, soybean, corn, cotton); and a petition process allowing for a determination that certain plants are no longer regulated articles.

Dr. Van Wert summarized the 84 comments received on the proposed rule. Fifty seven expressed general support and fifteen were opposed. Opposed comments criticized (1) the proposal to allow researchers to consult with Institutional Biosafety Committees (IBCs) regarding species other than the six explicitly mentioned in the rule; and/or (2) the lack of a waiting period before beginning a field test after notifying APHIS. APHIS responded by deleting the provision allowing for consultation with IBCs and adding provisions for waiting periods of ten days for movement and thirty days for introductions. Also added in the final rule was a provision which requires APHIS to notify states within five days of receiving a notification.

Dr. Van Wert reported that APHIS is working with the CSRS National Biological Impact Assessment Program to disseminate information on notifications and permits though an electronic database system. She concluded by noting that there are now four options for field testing genetically engineered organisms: (1) determining that the organism is not a regulated article; (2) using the notification system; (3) requesting a permit; or (4) petitioning for an

exemption from regulation. She distributed a list of notifications received and permits issued to date.

Dr. Andow asked how often the provisions for confidential business information (CBI) are used by applicants. Dr. Van Wert replied that this varies, and that a justification for using CBI must be attached to the application. She estimated that perhaps 50 percent of applications contain CBI.

Dr. Letourneau asked what can be done if researchers do not follow the performance standards after they notify APHIS and begin a field test. Dr. Van Wert replied that APHIS can still inspect the plot and halt the test if necessary.

Dr. Vidaver asked why notification is allowed for only six crops. She asked why squash is not included. Dr. Van Wert replied that other crops may be added to the notification list through a petitioning process with input from the public.

Working Group on Aquatic Biotechnology

Dr. Kapuscinski reported on the ABRAC Working Group on Aquatic Biotechnology and Environmental Safety which she chairs. She said the Working Group is organizing a Workshop on Performance Standards for Research with Genetically Modified Fish and Shellfish which will be held August 18-20, 1993, in Minneapolis, MN. USDA is sponsoring the workshop along with the Minnesota Legislature, Minnesota Sea Grant, and the University of Minnesota. She reported that the Working Group had met previously in October, 1992, to develop plans for the workshop and that the minutes of that meeting had been published.

The purpose of the Minneapolis workshop will be to provide a forum where a diverse group of people can comment in detail on the performance standards which are being developed by the ABRAC Working Group. Registrants for the workshop will receive the performance standards in advance. At the workshop, small groups will be assigned different portions of the standards for review and comment. After the workshop, the standards will be revised and then submitted to ABRAC for review by the full Committee. Dr. Kapuscinski explained the approach that the Working Group is taking in the development of the performance standards.

Dr. Vidaver commented on the term "ecological hazard" which Dr. Kapuscinski used in her description of the Working Group approach to the performance standards. Dr. Vidaver said she would prefer using a more neutral term such as "ecological concern."

Dr. Kapuscinski said that the term "ecological hazard " wasn't used in the draft performance standards, which at this time were actually a set of questions to be considered. Ms. Cordle added that the word "effects" was being used instead of "hazards".

Dr. Hill asked if attendance at the workshop will be limited. Dr. Kapuscinski replied that she did not wish to exclude anyone from participation, but she did wish to keep the group small enough to allow for discussion. Dr. Hill also asked if social scientists would be invited to attend. Dr. Kapuscinski said several bioethicists may attend. Ms. Cordle stated that the meeting has a very narrow focus, i.e., preparation of performance standards. Dr. Kline noted that social scientists could be helpful in determining the "value" of certain environments when discussing the effects of aquatic biotechnology.

Dr. Young noted that Dr. Kapuscinski represented the ABRAC and the United States as the keynote speaker at an Organization for Economic Cooperation and Development (OECD) meeting on environmental aspects of aquatic biotechnology in Trondheim, Norway in June, 1993. Dr. Kapuscinski said that many other countries have expressed an interest in the ABRAC workshop, and that some are likely to send representatives to the meeting. She added that the ABRAC activity dovetails nicely with the approach being taken by OECD.

Dr. Harlander requested an opportunity for the ABRAC to review the performance standards before the workshop. Dr. Kapuscinski said she would distribute them to the ABRAC two weeks before the meeting and that comments should be send to Ms. Cordle at OAB who will compile them.

The U.S. - EC Workshop on Biotechnology Communication

Dr. Young reported on the U.S. - EC Workshop on Methods of Communicating Biotechnology with the Public, which took place in March, 1992 in Dublin, Ireland. The workshop was an activity of the U.S. - EC Task Force on Biotechnology Research. It was attended by 40 specialists from the United states and member states of the European Community (EC). The purpose was to advise governments on effective means of communication. Dr. Harlander asked if there would be follow up activities stemming from the workshop. Dr. Young replied that some informational materials may be produced jointly.

Report on Survey and Focus Group Activities

Dr. Thomas Hoban, North Carolina State University (NCSU), reported on a phone survey and a series of focus group studies he conducted with Dr. Patricia Kendall of Colorado State University. The purpose of the focus group exercise was to gauge national public opinion about biotechnology. He said that final publication of the data from the survey is targeted for October, 1993. He said this ABRAC meeting provided the first public forum to discuss the results of the focus group studies.

Support for the survey and focus groups was provided by the USDA

Extension Service and NCSU. These activities had three objectives: (1) to better understand public opinion; (2) to inform public officials and other leaders about public opinion; and (3) to develop recommendations.

Dr. Hoban's telephone survey involved a random sample of 1228 people and it was conducted by professional interviewers. It had an estimated accuracy of 3 percent. The focus group exercise involved a smaller number of people who were organized into groups and invited to talk about their views on selected topics in biotechnology. Focus group exercises were conducted in Denver, Colorado and Raleigh, North Carolina and they involved groups that were both mixed and uniform by gender.

Dr. Kapuscinski asked how ethnically diverse the focus groups were. Dr. Hoban replied that the groups were not as diverse as he had hoped, but each group included at least one minority person.

Dr. Andow and Dr. Pierce asked how the participants were selected for the focus groups. Dr. Hoban replied that the process began with selection from the telephone book. Those who knew nothing of biotechnology were eliminated. He noted that focus group exercises differ from surveys in that participants are not selected totally at random. For example, volunteers for focus groups may already have a higher level of interest in the subject than the general public.

Dr. Hoban stated that some general patterns emerged from the telephone survey data. One pattern is that the degree of acceptance of biotechnology differs for different kinds of applications. Acceptance of interspecific gene transfer, for example, varies according the source and recipient of the genetic material. The most acceptable was plant to plant, while the least acceptable was human to animal.

Dr. Hoban said the focus groups allowed a more detailed examination of opinions, but were more subjective and subject to misinterpretation. Participants in the focus groups were asked to discuss four specific applications of biotechnology: (1) a tomato produced with antisense technology; (2) corn with insect resistance; (3) porcine somatotropin; and (4) transgenic fish. The response varied with least concern being expressed about the tomato and the most about the fish. He said the focus groups demonstrate that moral and ethical issues are significant to consumers and there is a high degree of interest in learning more about biotechnology.

Dr. Hoban added that the focus groups explored the types of information sources would be used by consumers including mass media, in-store brochures, toll-free telephone numbers, etc. He said the report of the focus groups will be available shortly.

Dr. Basu asked if the movie *Jurassic Park* will influence the public. Dr. Hoban replied that he believes the movie will stimulate public interest.

Dr. Van Wert asked how much consumers know about the food production system. Dr. Hoban replied that consumers generally believe food comes from the supermarket and they don't have a good understanding of the food and agricultural system.

Workshop on the Societal Issues of Food Biotechnology

Dr. Young described how the workshop that immediately preceded the ABRAC meeting came about. He recalled that a year ago the USDA Committee on Biotechnology in Agriculture (CBA) suggested that the topic of societal issues and food biotechnology needed to be examined. It was suggested that ABRAC, as an advisory committee, might be an appropriate vehicle for discussing this issue and beginning a dialogue with the public. The ABRAC accepted this issue as a project and approached the North Carolina Biotechnology Center about holding a meeting on the topic.

Dr. Young then asked the ABRAC to discuss what they heard at the meeting, and if there were any recommendations that they wished to pass on to the CBA. Dr. Kline noted that they ABRAC was no more likely to reach consensus about these issues, than were those who attended the meeting.

Dr. Harlander asked if the proceedings would be published. Steven Burke, North Carolina Biotechnology Center, said there would be published proceedings. Dr. Harlander said she came away from the meeting believing there is a real need for basic information by certain segments of the public, especially professional chefs and supermarket retailers. She said she could see a role for ABRAC and USDA in providing this information.

Dr. Kapuscinski expressed the view that the role of the Extension Service is very important in providing information. She said the role of agricultural extension is changing nationally and its mission is becoming broader. Dr. Young said the Extension Service has been involved in biotechnology education, but it really needs specific products of biotechnology in order to focus the attention of the public and the farming community.

Dr. Marrone reported that some microbial products are available for demonstration. Pest control advisers, she said, are eager to receive information on biotechnology, but they are not receiving much and, as a result, they are confused. Dr. Hoban expressed the view that the role of national extension in biotechnology will probably be limited. When agents request information, he said, they do so from state universities.

Dr. Wodzinski said there is a need to provide information through

the mass media. He advocated a professional television production on agricultural biotechnology, along the lines of public television's *Nova*, for the general public and for classroom use. The program should, he said, discuss today's agricultural system as well as scenarios for the future.

Dr. Letourneau stressed the importance of examining biotechnology from different perspectives, including non-scientific perspectives. She urged serious consideration of the larger ethical and societal implications of agricultural biotechnology and she commended the organizers of the North Carolina workshop on the societal implications of biotechnology for their efforts.

Dr. Kline identified two assumptions which may contribute to the ineffectiveness of public information programs on biotechnology. The first, he said, is the assumption that there is a single "public." It is more useful, he said, to consider many "publics," e.g., chefs, high school teachers, etc. Secondly, he claimed it was incorrect to think that information being provided by any information program is unbiased.

Dr. Vidaver said it would be useful for USDA and FDA to establish "800" numbers to answer questions about agriculture and food. She said it would be important to place transgenic organisms in the perspective of other organisms of agricultural importance.

Dr. Andow agreed with Dr. Kline that is important to realize that information is not neutral. He commended USDA and the North Carolina Biotechnology Center for organizing the conference on food biotechnology with a wide variety of viewpoints on the program.

Report on 3rd International Conference on Field Test Results

Ms. Steinbock briefed the Committee on the 3rd International Conference on Field Test Results planned for November, 1994 in Asilomar, California. [Staff note: The conference has since been rescheduled for Monterey.] Dr. Young expressed an interest in scheduling an ABRAC meeting in conjunction with the conference so that ABRAC members could more easlily participate. Ms. Steinbock invited ABRAC members to submit ideas for topics to be covered at the conference.

Report on Biotechnology Research in the 1890 Institutions

Dr. Hill reported that he was in the process of conducting a survey of the biotechnology research programs at the 1890 institutions. He distributed a partial list of these programs. He expressed the view that representatives of the 1890 institutions need to participate in ABRAC activities. He said USDA is committed to providing centers for excellence at these institutions and that ABRAC may have a role to play in the development of the centers which deal with biotechnology. At the next ABRAC meeting he said

he would make specific suggestions about increasing interactions between the ABRAC and the 1890 institutions.

Update on the Federal Biotechnology Research Initiative

Dr. Young briefed the Committee on the Federal biotechnology research initiative. He said the Fiscal Year (FY) 1994 report is being published and will be available soon. The White House, he said, has decided to scale back the biotechnology research initiative, at least for the coming year. The Federal Coordinating Council for Science, Engineering and Technology (FCCSET) Committee on Life Science and Health, he said, will study special issues involving biotechnology over the next few years.

Resumption of Discussion of Transgenic Animal Working Group Report

Dr. Lauderdale reported that he and Dr. Harlander, Dr. Kapuscinski, and Dr. Vidaver had condensed the Working Group report to six pages. The resulting draft is attached as Appendix D, #195b, and entitled "Recommendations to USDA by ABRAC on the Process to Assess Human Food Safety of Transgenic Animals, 29 June 1993."

Committee discussion centered on the suggested approach for determining human food safety (#195b, p. 4, sec. IIA) and on food safety testing strategies (#195b, p. 5, sec. IIA4).

Dr. Andow, in regard to sec. IIA4, raised a question about the relative value of *in vivo* and *in vitro* food safety testing stategies. Ms. Cordle referred to the approaches to new food safety evaluation taken by the U.S. Food and Drug Administration, the International Food Biotechnology Council, and the Organization for Economic Cooperation and Development. She recalled that all three organizations have proposed evaluating the safety of new biotechnology-derived foods relative to the corresponding traditional food. She advocated phrasing the food safety approach in terms of changes in the new food relative to the traditional food rather than in terms of the gene product alone. Dr. Lauderdale noted that sections IIA2 and 3 of #195b should capture the change in a new food relative to the traditional food.

Dr. Andow noted that the Committee had not yet agreed on the precise wording of #195b, sec. IIA, on the suggested approach for determining the human food safety of gene modifications. After extensive discussion and revision by the Committee, the text of #195b, sec. IIA read as follows: "The suggested approach for determining the human food safety of gene additions, gene modifications (e.g., transcription/translation, amplification, and deletion), mosaics, marker genes, and somatic cell therapy is as follows:"

Dr. Kapuscinski moved that the ABRAC recommend that USDA use Document 195b as a proposed scientific framework in its assessment

of human food safety of transgenic animals, and that OAB edit Document 195b to capture the comments made by ABRAC members at this meeting. Dr. Letourneau seconded the motion. After some discussion, the Committee approved the motion unanimously.

Dr. Young said that OAB would re-word appropriate sections of the document and distribute it to ABRAC members by telefacsimile for final review before forwarding it to the Assistant Secretary for Science and Education.

Dr. Norcross, FSIS, thanked the Committee for its efforts and he expressed support for circulating the revised document to the ABRAC members for one more round of review.

Transgenic Plants

Dr. Wodzinski expressed the view that the transgenic animal document turned out well, and he asked if a similar document was needed for transgenic plants. Dr. Vidaver expressed special concern for the disposition of plants that have been used in scientific research.

Ms. Cordle referred to the FDA policy statement of May 29, 1992 which addressed the safety of foods derived from new plant varieties. Dr. Vidaver asked if the FDA policy statement included plants used for research. Ms. Cordle replied that the FDA policy statement did not distinguish between experimental and commercial plants.

Future ABRAC Meetings and Working Groups

Dr. Kline posed the possibility of a new ABRAC working group in the education area. Dr. Harlander identified audiences that might be receptive to information on biotechnology including professional chefs, supermarket retailers, and high school teachers. Drs. Harlander, Marrone, and Pierce expressed interest in serving on an education working group. Steven Burke, North Carolina Biotechnology Center, provided members of the Committee with an information package on the educational activities of the Center. Dr. Young said that he will know more about the ABRAC funding situation in a few weeks and he mentioned the possibility of an ABRAC education working group meeting in October.

Dr. Harlander raised the question of whether there was a role for the ABRAC in the area of research priorities and research funding trends. Dr. Andow requested future feedback from OAB or other USDA offices on the feasibility of an ABRAC effort in the area of research priorities. Dr. Kline and Dr. Young said they would confer on those possibilities. Dr. Kelman said the USDA Biotechnology Council, which he chaired, may discuss a possible ABRAC role in research priorities and related areas of biotechnology research at its next meeting.

Dr. Young identified December 16-17, 1993 as the tentative dates for the next full ABRAC meeting.

Dr. Kline adjourned the meeting at 3:44 p.m.

MBSteinbock

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Revised:USDA:S&E:CSRS:OAB:DJones @ CJones:8/12/93

APPENDIX A

LIST OF VISITORS
UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL BIOTECHNOLOGY RESEARCH ADVISORY COMMITTEE
Meeting of June 29-30, 1993

Sandra Needham, Agriculture Canada
Frits van Vugt, Netherlands Ministry of Agriculture
Scott Shore, North Carolina Department of Agriculture
Sally Van Wert, USDA Animal and Plant Health Inspection Service
Daniel Adams, Allerx, Inc., Greenwich, CT

Margaret McEwan, Shaw's Supermarkets, E. Bridgewater, MA
Mary Ellen Jones, Virginia Polytechnic Institute
Barbara Masters, USDA Food Safety and Inspection Service
Eric Flamm, U.S. Food and Drug Administration
Pat Basu, USDA Food Safety and Inspection Service

Marvin Norcross, USDA Food Safety and Inspection Service
Steven Burke, North Carolina Biotechnology Center
Donna Maroni, North Carolina Biotechnology Center
Tong Wu, Chapel Hill, North Carolina
Gerald Messerschmidt, DNX, Inc., Princeton, NJ

Roger Straughan, University of Reading, U.K.
Ken Reid, *Food Chemical News*
Thomas Hoban, North Carolina State University
Steven Witt, Center for Science Information, San Francisco

Recommendations of the Transgenic Animal Working Group to the ABRAC on the Process to Assess Human Food Safety of Transgenic Animals
29 June 1993

Introduction

The Agricultural Biotechnology Research Advisory Committee (ABRAC) Transgenic Animal Working Group (henceforth referred to as the Working Group) met on April 8, 1993, in Room 3109 of the South Building of the U.S. Department of Agriculture in Washington, DC. Dr. James Lauderdale chaired the meeting. The meeting was open to the public and had been announced in the Federal Register of February 11, 1993 (58 FR 8034).

Working Group

Members of the Working Group in attendance were Dr. James Lauderdale, Dr. William Witt, Dr. Bennie Osburn, Dr. Susan Harlander, Dr. Ann Boyd, Dr. Richard Witter, Dr. Harold Hafs, Dr Gary Weber, and Dr. Duane Kraemer. Persons in attendance from the Office of Agricultural Biotechnology (OAB) were Alvin L. Young, Maryln K. Cordle, and Barry Stone. Persons from the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) who attended were Dr. Marvin Norcross, Dr. Barbara Masters, Dr. Pat Basu, Dr. Oto Urban, and Dr. Bharat Patel. Additional Visitors attending/participating numbered 22.

Status of Transgenic Animals and Human Food Safety

The FSIS had come before the ABRAC for assistance in June 1990 when ABRAC was asked to review criteria developed by FSIS for evaluating the food safety of non-transgenic animals produced from transgenic animal experiments, the so-called, "no

takes." The ABRAC reviewed and concurred with the proposed FSIS criteria. On December 17, 1991, FSIS published a notice entitled Livestock and Poultry Connected with Biotechnology Research (56 FR 67054). This notice announced the availability of a document entitled Decision Criteria for the Evaluation of Non-transgenic Animals from Transgenic Research.

Currently, FSIS requested that the Working Group address the human food safety issues associate with transgenic animals.

Human Food Safety Assurance

A report by the General Accounting Office (GAO) determined that 12 Federal agencies are involved with food safety. Among those agencies, FSIS ensures that meat and poultry products are safe, wholesome, and accurately labeled, as specified by the Federal Meat Inspection Act (FMIA) and the Poultry Products Inspection Act (PPIA). The U.S. Food and Drug Administration (FDA) is responsible for assuring the safety of food not inspected by FSIS. FDA also ensures that animal drugs are safe (particularly with respect to residues present in the animal at slaughter), effective, and properly labeled. The Center for Veterinary Medicine, FDA, sets tolerances for residues in food. Both FDA and FSIS ensure that feed additives used in meat and poultry are safe for consumers, and the two agencies have a close working relationship. USDA's Animal and Plant Health Inspection Service (APHIS) enforces the Virus Serum and Toxin Act and animal quarantine laws. Biologic products such as vaccines and serums used to protect the health of the animal are subject to APHIS oversight. The Environmental Protection Agency (EPA) controls and abates pollution in air, water, solid waste,

pesticides, radiation, and toxic substances. The agency also reviews and sets tolerances for residues of pesticide chemicals used directly on food animals or on animal feed crops before those chemicals are marketed and sets tolerances for the residues in food. Through a memorandum of understanding, USDA, FDA and EPA coordinate their activities in the regulation of human food safety.

Approach to Regulating Transgenic Animals

The proposed definition of transgenic animals is as follows:

> *Transgenic animals, for the purpose of this document, are animals in which foreign DNA is detected.*

FSIS noted that this definition addresses human food safety, not gene expression *per se*. The Working Group supports the concept of addressing human food safety of food derived from transgenic animals. Based on the discussion of the Working Group members and input from attendees, the Working Group recommends the following approach to assessing human food safety of transgenic animals.

I. Non-Transgenic Animals

The Working Group agreed that the first question to pose would be: "Is the animal in question transgenic?" The animal will be declared to be not transgenic based on the criteria described in the Federal Register notice of

December 27, 1991 (56 FR 67054); such non-transgenic animals may enter the human food chain without further action in accord with 56 FR 67054.

II. Transgenic Animals

Animals will be declared to be transgenic based on at least one of the following: 1) detection of the transgene by Southern hybridization or polymerase chain reaction or other appropriate methods, or 2) presence of measurable gene product, or 3) presence of transgene associated traits. The level of detection is one copy of the foreign DNA per haploid genomic equivalent by DNA hybridization techniques.

A. The suggested approach for determining the human food safety of gene products.

1. Identify the gene product, the concentration of the product, and the tissue distribution (per edible tissue of concern in determining food safety).

2. If the gene product is endogenous:

a. Review both the technical literature and the regulatory history for that gene product and regulate based on existing human food safety guidelines, e.g. FDA/FSIS

guidelines.

b. If there is no or limited technical literature and no or limited regulatory history for that gene product, convene an expert panel to define the assays/animal models/sensitivity/specificity questions that must be addressed in order to determine human food safety.

3. If the gene product is non-endogenous, identify the expressed product. Questions posed should be based on the type of product identified. Use of existing guidelines (e.g. FDA/FSIS guidelines) is recommended to start. If those guidelines are not adequate, convene an expert panel to develop appropriate guidelines.

a. Perform assay validations for sensitivity and specificity with respect to food safety requirements.

b. Identify the human food safety concerns associated with *in vivo* (surrogate and host animals) and *in vitro* assays using appropriate methodology. The unintended effect of a promoter-enhancer <u>cis</u> sequence used to express the desired gene should be tested *in vivo* and *in vitro* by assays for the gene product. Secondary effects of enhancers on distant endogenous genes should be evident

in model systems in order to predict deleterious side effects. The orientation of the promoter to the inserted gene during *in vitro* cloning is subject to experimental design. The cloning vectors have specific positions in which the gene and its regulatory elements are placed in ways that predict the successful expression of the gene product. However, enhancer elements influence gene expression from distal sites in both directions and in positive and negative ways in different cell types. Therefore, some testing strategy should be included in proposals that assay or detect unwanted effect of host genes.

c. If the current FDA/FSIS guidelines are not adequate, convene an expert panel to suggest methods and data requirements for any gene product that has too small a database to make a human food safety decision.

4. Determine secondary influences.

a. Determine if the DNA transferred is targeted to the gene insertion site.

b. Determine by visual and traditional pathology and

toxicological testing methods if there are any secondary effects (pleiotropic effects) of food safety concern related to the gene insertion site.

c. Determine if there are metabolic effects of the gene product, which are of human food safety concern (suggest use of existing FDA/FSIS guidelines).

d. If a marker gene is included to track the exogenous recombinant DNA molecule, the marker gene and its product(s) is (are) subject to the same criteria as for transgenes because the marker gene is also foreign and presumably will be transferred with the exogenous elements into the host DNA.

B. The suggested approach for "naked DNA" and "marker genes."

1. Determine if the DNA is physically integrated into the genome.

a. If "Yes," proceed with the recommendation for gene products (A).

b. If "No," the time of disappearance must be determined. If it is not infectious to humans, then there is no concern

about human food safety. If it is infectious to humans, animal products of these transgenic animals should be prohibited from entering the human food chain.

2. If the DNA is a chemically modified nucleic acid such as synthetic oligonucleotides with stabilizing modifications to nucleotides or to the backbone, etc.

   a. If "No," there is no concern about human food safety.

   b. If "Yes," address human food safety by assessing the stability, infectivity and expression. If there is no stability, no infectivity, and no expression, there is no human food safety concern. If there is expression, or infectivity, or stable incorporation, human food safety should be addressed through exclusion of food products from human consumption until a panel of experts is convened and devises guidelines to assure human food safety.

   c. If naked DNA is used for vaccine or other purposes such as enclosed in synthetic vesicles or viral coats, then assays are needed to determine if the DNA remains in an "infectious" form. If "No," then the food product is safe. If

"Yes," exclude food products until safety is determined by a panel of experts who would devise appropriate guidelines.

C. The suggested approach for gene amplification and deletions.

Amplifications and deletions could be addressed by answering the questions developed for gene products (A).

1. Amplification of exogenous genes by use of expression vectors designed to include DHFR which amplifies during host DNA replication and thereby includes the foreign element in amplification are used to insure enhanced gene expression. These designer vectors, therefore, should be tested for level of product expression and for copy number by the same sensitive DNA hybridization techniques used in determining transgenics (II).

2. Deletions may occur through random illigimate recombination of the foreign DNA into the host genome thereby inactivating a functioning allele. Such random integration is the expected effect of non-targeted gene therapy at the embryonic and somatic cell level. Detection of the integration site would predict gene "knock-out" effect(s) and any negative side-effect(s) on the transgenic animal. Strategies for gene targeting at specific chromosomal loci

are not expected in transgenic production. Therefore, one must assume the norm is integration of foreign DNA at random positions, thus, transgenic animals should be examined for deleterious side effects of random integration.

D. The suggested approach for viral vectors

Viral vectors have been approved safe for clinical trials in animals and humans in somatic cells but not in embryonic tissue. Viral vectors are an alternative method of introducing foreign DNA into host cells in somatic therapy and in embryonic stem cells which, when incorporated into the developing embryo, result in chimerics or mosaic animals.

1. Viral vectors include a wide range of agents. Small DNA viruses whose insertion sequence along with viral DNA integrates into the host chromosome without further virus replication should be treated as transgenics and evaluated the same.

2. Cytoplasmic virus vectors, e.g., vaccinia, should be tested for appropriate expression of the exogenous gene product at levels appropriate for the gene product (A). The guidelines for safety should include determination of spread or infectivity of the virus and effect of the gene product (A) on the food product.

3. Retroviral vectors are designed to deliver foreign DNA into the host cell chromosomes. When used in embryonic tissue and integration occurs throughout the animal, the animal is by definition transgenic and should be regulated as such. When used in somatic cell therapy, the mosaic criteria should be applied. When specific proposals are submitted for use of a retroviral vector as a gene transfer agent, human food safety should be evaluated according to specific guidelines of an expert panel because recombination with endogenous viral sequences may require further evaluative criteria than those used for transgenics.

4. Viral enhancers have the potential to influence host cell genes near their insertion site and should be evaluated as stated for expression vector enhancers above.

E. The suggested approach for mosaics.

A mosaic is an animal in which the foreign DNA has inserted into some cells of the animals and not in all. Throughout development, the animal has a hybrid character of being transgenic in selected tissues and not in others. Such animals may fail to test positive as transgenics since the foreign DNA is not present in all cells or tissues. Breeding of mosaics for several generations can produce occasional transgenics and,

therefore, animals in subsequent generations should be tested for transgenic according to criteria stated in, Transgenic Animals, Safety of Gene Products (II. A).

F. The suggested approach for antisense and somatic cell therapy.

1. The antisense gene should be detected according to criteria for detection of foreign DNA and then treated as transgenic or not.

2. Expression of the antisense and specific target gene repression should be evaluated according to level of activity of the target gene product.

3. The therapeutic transient use of antisense therapy should be evaluated according to specificity of the antisense genetic target's level of expression, time of effect, stability of the antisense nucleic acid, and potential for integration. Without integration, the agent should be evaluated according to naked DNA (B) or viral transfer (D) criteria and, if integrated, by criteria for transgenics (A).

4. If the gene is integrated, there is no human food safety concern with respect to the gene itself.

G. Scope of Safety Evaluation.

The Working Group agreed that the human food safety evaluation should address the safety of food products from animals genetically modified by deliberate human intervention, but should exclude traditional animal breeding.

JWL/cat
21 June 1993

Appendix C

DECISION CRITERIA FOR THE EVALUATION OF NONTRANSGENIC ANIMALS FROM TRANSGENIC ANIMAL RESEARCH

UNITED STATES DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE
SCIENCE AND TECHNOLOGY
WASHINGTON, DC 20250
(202) 720-8623

1991

Decision Criteria for the Evaluation of Nontransgenic Animals From Transgenic Animal Research

Connie L. Bacon, D.V.M., Residue Evaluation and Planning Division, U.S. Department of Agriculture, Food Safety and Inspection Service, 300 12th Street, S.W. Washington, DC 20250*

Tremendous advances have been made in livestock production in the last two decades. Artificial insemination and embryo transfer are now routine procedures in many cattle operations. Biotechnology promises even more advances in the production of livestock than artificial insemination or embryo transfer. In the near future it may be possible to develop commercially important changes in livestock in one generation that would have previously taken many years to accomplish through traditional selective breeding practices. Presumably the genetically engineered livestock of the future will provide producers with more efficient animals and consumers with products that were produced using fewer feed additives, are lower in fat and cholesterol and contain fewer pharmaceutical, biological and chemical residues.

In 1982, the first transgenic animals that expressed the injected foreign DNA were produced. These mice, containing a rat growth hormone gene linked to a

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metallothionein promoter, are probably the best known transgenic animals produced to date. Thousands of transgenic mice have been produced since that time, with a wide variety of transgenes and for many different purposes. In 1985 the first attempts were made to develop transgenic livestock using the same methods employed in mice. The results were very disappointing and it is now recognized that the production of transgenic cattle, sheep, goats, and swine is far more difficult than the production of transgenic mice.

There appear to be four major reasons that transgenic livestock are so difficult to produce. First, in murine eggs the pronuclei are readily visible, making microinjection a fairly simple procedure. On the other hand, the cytoplasm of the larger mammals is very dense and special techniques must be employed in order to visualize the pronuclei. These techniques often reduce the viability of the embryos. Second, the recovery of suitable eggs for microinjection is more difficult. Fewer eggs per donor female are obtainable in livestock. Third, optimum *in vitro* culture conditions have yet to be established for pig, sheep, and cattle eggs through morula or blastocyst stages. The fourth factor that makes transgenic livestock more difficult to produce than mice is the long generational interval. The gestation of pigs, sheep, goats, and cattle range from 114 to 283 days with the onset of puberty ranging from six months to two years. This can be contrasted to the mouse which has a gestation of 20 days and a 28-day onset to puberty.

The efficiency of producing transgenic mice has been reported to approach 20% of the injected ova. The efficiency of producing transgenic livestock is less than 2%. Due to this extreme inefficiency and the economics of housing,

maintenance, and equipment required to produce transgenic livestock, it has been estimated that the average cost of producing a transgenic cow approaches \$1 million. Therefore, due to the large number of nontransgenic animals which result from transgenic animal experiments and the high cost of maintaining these animals, it is not surprising that researchers are interested in slaughtering the nontransgenic animals which are a product of these types of experiments.

The production of mosaic transgenic animals is often a complication for researchers working with transgenic mice. Mosaics are animals which do not contain the transgene in all cells of the body. This is thought to occur in mice fairly often because of the timing of fertilization and microinjection of the one-cell embryo. It is hypothesized that the chromosomal DNA has already begun to replicate at the time of microinjection. In the production of transgenic livestock, the frequency of occurrence of mosaic animals is very low when one-cell embryos are microinjected. This is most likely due to the longer development time of the larger animals compared to mice. The DNA is not replicating as quickly in the larger mammals in this early stage and first cleavage occurs much later than it does in mice.

The mosaic mice which have been produced through microinjection techniques are uniformly mosaic in the somatic cells. Therefore, all organ systems possess approximately the same percentage of cells containing the transgene.

Mosaic transgenic animals can also be produced by using a viral vector as the means of introducing the transgene into an embryo which is usually at the 8-16

cell cleavage stage. Mosaicism in this situation is the desired outcome of the researcher and is a technique often employed to study gene expression.

A variety of methods are used to test the animals which result from transgenic animal experiments for the presence of the transgene. The two most commonly used methods are Southern Hybridization and the Polymerase Chain Reaction (PCR). Both of these methods can be highly specific and highly sensitive under the proper conditions. The sensitivity and specificity are based on the knowledge of the precise DNA sequence which was introduced into the embryo.

The amount of genomic DNA needed to generate a detectable hybridization signal using the Southern Hybridization method depends on a number of factors. These include the proportion of the genome that is complementary to the probe, the size of the probe and its specific activity, and the amount of genomic DNA transferred to the filter. Under optimum conditions, this method is capable of detecting 0.1 pg of DNA complementary to the probe with an autoradiographic exposure of several days. Therefore, a sequence 1000 bp in length that occurs only once in the genome (1 part in 3 million in mammals) can be detected on an overnight exposure, if 10 ug of genomic DNA is transferred to the filter and hybridized to a probe several hundred nucleotides in length. The use of PCR to amplify the gene sequence of interest allows the detection by Southern Hybridization of a single copy gene to be detected in the presence of a 10^{13}-fold excess of irrelevant DNA.

These two methods, Southern Hybridization and PCR, can then be used to test for the presence of the transgene in animals. Southern Hybridization and PCR

are also capable of detecting mosaic animals. These techniques are able to detect one copy of the transgene in 10% or fewer of the animal's cells. The most extreme mosaic reported to date is a mouse with the transgene present in 15% of its cells. The failure to detect the transgene by these methods supports the conclusion that the foreign DNA failed to incorporate into the genome of the animal.

The criteria that may be used to determine if the animals are nontransgenic are: 1) failure to detect the presence of the transgene by Southern Hybridization, the Polymerase Chain Reaction, or other appropriate scientific methods, 2) absence of a measurable gene product, 3) absence of transgene-associated traits, and 4) a healthy appearance.

The Food Safety Inspection Service (FSIS) has concluded that these nontransgenic animals (determined by some or all of the above methods) can be slaughtered safely under Title 9, Code of Federal Regulations (CFR) Sections 309.17, Livestock Used In Research. Animals exposed to a viral vector require prior approval by the Animal and Plant Health Inspection Service (APHIS). Under 9 CFR 309.17, the researcher must submit an application for slaughter to FSIS. This application must contain data demonstrating the methods employed to differentiate transgenic animals from nontransgenic animals. The application must also include such information as the number, age, sex, and identifying marks, such as tatoos or eartags, of the animals proposed for slaughter, and pharmaceuticals, biologics, or chemicals the animals were administered and the last date of administration, and the proposed date and establishment of slaughter. Upon receipt of all necessary information, FSIS

will review and evaluate the application. Provided that all criteria outlined in 9 CFR 309.17 are met, the animals described in the application will be approved for slaughter. These animals are identified upon presentation to the USDA Inspector-In-Charge at the slaughter plant as having been involved in research. They are maintained as a separate lot throughout the slaughter procedure. If the animals appear normal on antemortem and postmortem inspection they are passed for human consumption.

In addition to the research currently being conducted with transgenic livestock, there is also active research aimed at producing transgenic poultry. The production of transgenic poultry poses some unique problems for researchers, but the food safety of these birds would be assessed in a similar manner as transgenic livestock. If the birds are shown to be nontransgenic, then they may be eligible for slaughter under Title 9, CFR 381.75, Poultry Used in Research. The requirements of this regulation mirror those of 9 CFR 309.17.

FSIS feels that it is appropriate to slaughter these nontransgenic animals under 9 CFR 309.17 as livestock used in research or 9 CFR 381.75 as poultry used in research. On June 22, 1990, FSIS presented the proposed decision criteria for the slaughter of nontransgenic animals from transgenic animal research to USDA's Agricultural Biotechnology Research Advisory Committee (ABRAC). ABRAC unanimously voted to endorse the process by which FSIS will evaluate and present for slaughter such animals produced in the course of transgenic animal research.

Animals which are shown to contain the transgene by Southern Hybridization and/or the Polymerase Chain Reaction must be evaluated separately. FSIS is currently in the process of drafting a statement of the process which will be used to evaluate the food safety of meat, poultry, and meat and poultry products derived from products of biotechnology. FSIS plans to present this statement to ABRAC.

September 1990

Slightly revised December 1991, August 1992

§ 309.17 Livestock used for research.

(a) No livestock used in any research investigation involving an experimental biological product, drug, or chemical shall be eligible for slaughter at an official establishment unless:

(1) The operator of such establishment, the sponsor of the investigation, or the investigator has submitted to the Program, or the Veterinary Services unit of the Animal and Plant Health Inspection Service of the Department of Agriculture or to the Environmental Protection Agency or to the Food and Drug Administration of the Department of Health, Education, and Welfare, data or a summary evaluation of the data which demonstrates that the use of such biological product, drug, or chemical will not result in the products of such livestock being adulterated, and a Program employee has approved such slaughter;

(2) Written approval by the Deputy Administrator, Meat and Poultry Inspection Field Operations is furnished the area supervisor prior to the time of slaughter;

(3) In the case of an animal administered any unlicensed, experimental veterinary biologic product regulated under the Virus-Serum Toxin Act (21 U.S.C. 151 et seq.), the product was prepared and distributed in compliance with Part 103 of the regulations issued under said Act (part 103 of this title), and used in accordance with the labeling approved under said regulations;

(4) In the case of an animal administered any investigational drug regulated under the Federal Food, Drug, and Cosmetic Act, as amended (21 U.S.C. 301 et seq.), the drug was prepared and distributed in compliance with the applicable provisions of part 135 of the regulations issued under said Act (21 CFR part 135), and used in accordance with the labeling approved under said regulations;

(5) In the case of an animal subjected to any experimental economic poison under section 2(a) of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (7 U.S.C. 135 et seq.), the product was prepared and distributed in accordance with § 362.17 of the regulations issued under said Act (7 CFR 362.17), and used in accordance with the labeling approved under said regulations.

(6) In the case of an animal administered or subjected to any substance that is a food additive or pesticide chemical under the Federal Food, Drug, and Cosmetic Act, supra, there has been compliance with all tolerance limitations established by said Act and the regulations promulgated thereunder (21 CFR 1.1 et seq.), and all other restrictions and requirements imposed by said Act and said regulations will be complied with at the time of slaughter.

(b) The inspector in charge may deny or withdraw the approval for slaughter of any livestock subject to the provision of this section when he deems it necessary to assure that all products prepared at the official establishment are free from adulteration.

§ 381.75 Poultry used for research.

(a) No poultry used in any research investigation involving an experimental biological product, drug, or chemical shall be eligible for slaughter at an official establishment unless the operator of such establishment, the sponsor of the investigation, or the investigator has submitted to the Inspection Service, or the Veterinary Biologics unit of Veterinary Services, Animal and Plant Health Inspection Service of the Department or the Environmental Protection Agency, or the Food and Drug Administration of the Department of Health, Education, and Welfare, data or a summary evaluation of the data which demonstrates that the use of such biological product, drug, or chemical will not result in the products of such poultry being adulterated, and the Administrator has approved such slaughter.

[37 FR 9706, May 16, 1972, as amended at 39 FR 4569, Feb. 5, 1974]

D.J. 195b

Appendix D

Recommendations to USDA

by ABRAC on the

Process to Assess Human Food Safety of Transgenic Animals

29 June 1993

ABRAC recommends that FSIS/USDA capture the descriptive text of document 194a and 195a for future use in addressing this topic.

Introduction

The Agricultural Biotechnology Research Advisory Committee (ABRAC) Transgenic Animal Working Group (henceforth referred to as the Working Group) met on April 8, 1993, in Room 3109 of the South Building of the U.S. Department of Agriculture in Washington DC. Dr. James Lauderdale chaired the meeting. The meeting was open to the public and had been announced in the Federal Register of February 11, 1993 (58 FR 8034).

Working Group

Members of the Working Group in attendance were Dr. James Lauderdale, Dr. William Witt, Dr. Bennie Osburn, Dr. Susan Harlander, Dr. Ann Boyd, Dr. Richard Witter, Dr. Harold Hafs, Dr. Gary Weber, and Dr. Duane Kraemer. Persons in attendance from the Office Agricultural Biotechnology (OAB) were Alvin L. Young, Marilyn, K.. Cordle, and Barry Stone. Persons from the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) who attended were Dr. Marvin Norcross, Dr. Barbara Masters, Dr. Pat Basu, Dr. Oto Urban, and Dr. Bharat Patel. Additional visitors attending/participating numbered 22.

Status of Transgenic Animals and Human Food Safety

The FSIS had come before the ABRAC for assistance in June 1990 when ABRAC was asked to review criteria developed by FSIS for evaluating the food safety of non-transgenic animals produced from transgenic animal experiments, the so-called, "no takes." The ABRAC reviewed and concurred with the proposed FSIS criteria. On December 17, 1991, FSIS published a notice entitled Livestock and Poultry Connected with Biotechnology Research (56 FR 67054). This notice announced the availability of a document entitled Decision Criteria for the Evaluation of Non-transgenic Animals from Transgenic Research.

Currently, FSIS requested that the Working Group address the human food safety issues associated with transgenic animals

Human Food Safety Assurance

A report by the General Accounting Office (GAO) determined that 12 Federal agencies are involved with food safety. Among those agencies, FSIS ensures that meat and poultry products are safe, wholesome, and accurately labeled, as specified by the Federal Meat Inspection Act (FMIA) and the Poultry Products Inspection Act (PPIA). The U.S. Food and Drug Administration (FDA) is responsible for assuring the safety of food not inspected by FSIS. FDA also insures that animal drugs are safe (particularly with respect to residues present in the animal at slaughter), effective, and properly labeled. The Center for Veterinary Medicine, FDA, sets tolerances for residues in food. Both FDA and FSIS ensure that feed additives used in meat and poultry are safe for consumers, and the two agencies have a close working relationship. USDA's Animal and Plant Health Inspection Service (APHIS) enforces the Virus Serum and Toxin Act and animal quarantine laws. Biologic products such as vaccines and serums used to protect the health of animals are subject to APHIS oversight. The Environmental Protection Agency (EPA) controls and

abates pollution in air, water, solid waste, pesticides, radiation, and toxic substances. The agency also reviews and sets tolerances for residues of pesticide chemicals used directly on food animals or on animal feed crops before those chemicals are marketed and sets tolerances for the residues in food. Through a memorandum of understanding, USDA, FDA and EPA coordinate their activities in the regulation of human food safety.

Approach to Regulating Transgenic Animals

FSIS is requested by ABRAC to write a preamble that addresses the context of current guidelines for human food safety. The Working Group agreed that the human food safety evaluation should address the safety of food products from animals genetically modified by deliberate human interventions, but should exclude traditional animal breeding. The proposed definition of transgenic animals is as follows:

For the purposes of this document, transgenic animals are defined as animals and their progeny whose genetic composition has been changed by introducing specific genes (e.g. recombinant DNA). It is intended that the scope of this definition for transgenic animals will also include antisense, amplification and deletion technologies.

This definition addresses human food safety, not gene expression *per se*. These recommendation cover transgenic animals as well as animals treated with somatic and other cell therapy. ABRAC supports the concept of addressing human food safety of food derived from transgenic animals. Based on the discussion by ABRAC of the Working Group recommendations and input from attendees at the ABRAC meeting, ABRAC recommends the following approach to assessing human food safety of transgenic animals.

I. Non-Transgenic Animals

Animals will be declared to be not transgenic based on the criteria described in the Federal Register notice of December 27, 1991 (56 FR 67054); such non-transgenic animals may enter the human food chain without further action in accord with 56 FR 67054.

II Transgenic Animals

Animals will be declared to be transgenic based on at least one of the following: 1) detection of the transgene by Southern hybridization or polymerase chain reaction or other appropriate methods, or 2) presence of measurable gene product, or 3) presence of transgene associated traits. The level of detection is one copy of the foreign DNA per haploid genomic equivalent by DNA hybridization techniques. If the DNA is infectious to humans the animal products of these transgenic animals must be demonstrated to be safe for human consumption.

A. The suggested approach for determining the human food safety of gene additions, or gene modifications, ~~of~~ (e.g. transcription/translation, amplification ~~(i.e. antisense)~~, and deleting or mosaics, (i.e., antisense) and of marker genes) and of somatic cell therapy, is as follows:

1. ~~Identify~~ Detect the gene product, the concentration of the product, and the tissue distribution (per edible tissue of concern in determining ~~of~~ safety). ~~If the conc & distr~~ [illegible] gene prod. are not different from non-trans animal, the trans animal will be used for human food. (JL)

2. Review both the technical literature and the regulatory history for that gene product and regulate based on existing human food safety guidelines, e.g. FDA/FSIS guidelines

If conc + distr. of gene prod. are not diff from those of non-trans animal, [illegible] AS. (PM)

[on HFS]

3. If there is no or limited literature and no or limited regulatory history for that gene product, convene and expert panel to define the assays/animal models/sensitivity/specificity questions that must be addressed in order to determine human food safety.

4. Identify the human food safety concerns ~~associated with~~ using preferably *in vivo* (~~surrogate and host animals~~) ~~and *in vitro*~~ assays, ~~using appropriate methodology~~. The unintended effect of a promoter-enhancer cis sequence used to express the desired gene should be tested preferably *in vivo* ~~and~~ or ~~*in vitro*~~ by assays for the gene product. Secondary effects of enhancers on distant genes should evaluated in the host or in scientifically defensible model systems in order to identify deleterious side effects. ~~The orientation of the promoter to the inserted gene during in vitro cloning is subjected to experimental design. The cloning vectors have specific positions in which the gene and its regulatory elements are placed in ways that predict the successful expression of the gene product. However, enhancer elements influence gene expression from distal sites in both directions and in positive and negative ways in difference cell types.~~ Therefore, some testing strategy should be included in proposals that assay or detect unwanted effect of host genes.

5. Determine secondary influences.

5d

a. Determine by visual and traditional pathology and toxicological testing methods if there are any secondary effects (pleiotropic effects) of food safety concern.

b. Determine if there are metabolic effects of the gene product, which are of human food safety concern (suggest use of existing FDA/FSIS guidelines.)

c. If a marker gene is included to track the recombinant DNA molecule, the marker gene and its product(s) is (are) subject to the same criteria as above because the marker gene presumably will be transferred into the host DNA.

B. The suggested approach for viral (and other) vectors

1. The guidelines for safety should include determination of spread or infectivity of the virus/viroids/other vectors. Additionally, the criteria of Section II A should be applied to address human food safety.

2. When specific proposals are submitted for use of a retroviral vector as a gene transfer agent, human food safety should be evaluated according to specific guidelines developed by an expert panel because recombination with endogenous viral sequences may require further evaluative criteria than those used for transgenics. When retroviral vectors are used in somatic cell therapy, the criteria of Section II A should be applied to address human food safety.

3. Viral enhancers have the potential to influence host cell genes near their insertion site and should be evaluated as stated for expression vector enhancers above.